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Computational Modeling of Laser-Cell Biochemical Interactions

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14. ABSTRACT
A computer-based model has been developed to explore the effects of lasers on the eye. The model was developed using a technique known as BioFusion®, which embodies a software engineering and knowledge acquisition methodology. The technique provides a formal means for developing a model by gathering, organizing, analyzing, documenting, synthesizing, and implementing the vast amount of knowledge needed about a biological process into a working computer simulation. The resulting PC-based model can then be used to explore questions in an *in silico* environment, and to work synergistically with in vitro and in vivo experiments.

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TABLE OF CONTENTS

LIST OF FIGURES

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1.0 INTRODUCTION

1.1 Background/Scope/Program Objectives

Any biological process is extremely complex, involving numerous cell types in a variety of states, as well as extracellular molecules and cytokines. These biological processes have extensive interconnections and complicated feedback loops that help to produce stable, self-repairable systems. In a healthy, "normal" biological system the multitude of processes are in a homeostatic state. However, should an event occur, such as an injury or infection, this stable state is automatically modified to bring about changes to address the problem. Eventually, if the response is successful, the injury is healed or the infection is cleared, and the biological system can return to a healthy, "normal" homeostatic state. However, even when the response is successful, the healing/repair process is not always perfect and often results in modifications to the specific environment, such as fibrosis and loss of functionality. In many areas of the body such imperfect healing simply results in a blemish on the skin, a welt in a bone, or a less flexible joint. However, in some locations, such as the heart or the eye, imperfect repair can result in significant loss of capability such as heart disease or blindness.

A biological system, as with any complex system, can be broken down hierarchically so that different views on the system can be addressed. For example, the typical breakdown of the human system includes the digestive system, the circulatory system, the skeletal system, the muscular system, the respiratory system, the nervous system, etc. These lines are somewhat artificial, but drawing such divisions allows us to deal with the extreme complexity of biological systems. Within each system are specific organs and processes that perform the functions required of that particular system. In the case of the nervous system, such components include the brain, the spinal cord, and the senses. Thus, the eye is considered part of the nervous system as it provides visual input to the brain. The eye is an extremely complex organ, involving numerous layers of varying cell types to perform its function of converting photons into a signal that can be interpreted by the brain as a visual image. In humans this capability is highly evolved and sight is an extremely important source of information about the world around us.

The ultimate goal of any work in developing a model of the eye would be to build a complete model of the cells and processes found in the eye, especially the retina, as it is the primary facility for sensing light and converting them to sensory signals. Building such a model would require considerable resources and time to try to do all at once. Addressing such complexity would be best handled by building a modular model that can address one component at a time, allowing the entire model to be built one manageable chunk at a time. Because the interest of the AFRL711 HPW/ RHDO is the effect of lasers on the eye, and in particular the thermal, photochemical, and photomechanical effects on the retina, the area focused on in this project was the development of a computational model of the complex cellular interactions that occur in the retina of the eye when exposed to a laser, with particular emphasis on the retinal pigmented epithelial cells, or RPEs. This model emphasizes the control systems and feedback loops internal to the RPE that are involved in the cellular response to such exposure, utilizing any specific data available about how RPEs respond to various levels of laser exposure. Of

course, the response of RPEs to such exposure invokes an entire cascade of events involving a typical biological system's response to stress, including the biochemical reactions that alter cell states and the call for cells from the local area and blood stream in response to such stimulation. Portions of these interactions were also modeled based on their direct affect on the RPEs. Thus, the model also contains high-level knowledge of the various cellular responses to the inputs, and the cell-to-cell interactions based on their cytokine and other chemical output. Input to the model allows time-history of ambient temperature, low-light exposure levels, and antioxidant availability. A primary output of the model involves levels of RPE viability over time, as well as the time course of many relevant values available about the RPE and the processes taking place in it.

The modeling approach used in the work reported on here is a technique called BioFusion®[1], which was developed by the Principle Investigator, and for which she is co-inventor on two patents. This is a unique, advanced modeling technique that can integrate a variety of levels of modeling, from numerical to symbolic, into a single model, allowing the ability to model large, complex sets of cellular and chemical interactions on a PC-based computer. Models can be built from the sub-cellular level up through entire disease processes. The approach allows for a systematic collection, organization, and analysis of the knowledge needed to build the model, as well as a modular, structured approach to building the computational model using a commercial, off-the-shelf product.

Current research in the effects of lasers on the eye have centered on developing an understanding of how light and heat affect the various cells in the eye, particularly the RPEs, and the level of exposure required to produce a certain short-term effect on the RPEs. Mathematical models have been developed (Torres et al., 1993; Till et al., 2003; Chen et al, 2006; Goldberg et al., 2007) that represent these effects. The BioFusion approach used in the work reported on here produces a higher-level model of the problem, modeling a large portion of the RPE cell, including its "normal" healthy state and how its behavior is modified due to various levels of laser exposure. This involved modeling various intra-cellular processes, including the internal biochemical cascades that are initiated under light exposure, plus metabolic and redox processes, and the effect of oxidative stress on the cell's lipoproteins, proteins, and DNA. This approach to modeling allows researchers to integrate the findings from the mathematical approaches to laser damage that are available, including thermal, photochemical, and photomechanical effects on the RPEs, with the knowledge of the RPEs' behavioral response to such exposure, as well as the cellular interactions occurring in the RPEs' immediate environment. Use of the modular, hierarchical approach to developing the model allowed work to focus on the exploration of the effects on the RPEs' viability and later to expand the model to include details of other cell types and processes found in the eye, and their reactions, to such exposure.

1.2 Modeling Biological Processes

Knowledge about biological processes is inherently complex, variable, and uncertain. The levels of knowledge needed to understand how a biological process is influenced are: 1) sub-cellular, chemical interactions that generate a local environment, 2) individual cell behaviors

[1] BioFusion is a registered trademark of Interleukin Genetics, Inc.

within an environment, 3) sets, or pools, of a specific cell type within an environment, 4) aggregations of different cell pools whose chemical products and behaviors create a particular environment, and 5) the environment generated by the aggregation of cell pools that manifests itself at the system, organ, or patient level as the disease's symptom complex. This list contains a feedback loop, since the environment listed in number one is aggregated to generate a global system environment in number four, which in turn influences numbers two and three, and is manifested at the system/organ/patient level in number five as the symptom complex. The complex interaction between the levels of biological knowledge makes it advantageous to represent and implement the knowledge independently within a hierarchy to accurately specify all relationships between levels (Fink and Herren, 1996).

Thus, in order to model a complex biological process, such as the processes that are initiated in the RPEs by exposing the eye to a laser, sufficient detail must be provided so that the model can run as a simulation. For example, sufficient knowledge must be available about normal RPE cellular behavior, the specific initial effects laser exposure has on the RPE individually, what response these cells have to those specific initial effects, what signals RPEs send externally that would affect other RPEs (and other cell types) in the area, what possible states the RPEs can be in based on their level of exposure to the laser, what environmental factors might affect the cells and their response to laser exposure, and what intra-cellular processes get initiated as a result of the direct exposure as well as the feedback from the environment generated by the stimulated/damaged RPEs.

Methods of acquiring and representing such knowledge about biological processes to build a viable model must surmount a number of obstacles and issues. These are as follows:

1) *The level of existing knowledge about a disease/repair process and the basic supporting biology tends to be quite shallow.* Researchers often do not have information about exact causal relationships and only have empirical data on associations between stimulus and resulting behaviors.

2) *Anatomy and physiology of a particular system is complex and variable.* Biological processes have a large amount of variation. Not only do aspects of anatomy vary from person to person but the basic biological processes are greatly modified by the background, history, and genetics of a particular patient. The quantity of knowledge that must be acquired and encoded can be huge, so efforts must be made to simplify and aggregate wherever possible and appropriate. In addition, space/distance often plays an important role in how various portions of the anatomy interact and influence each other in a particular biological process.

3) *Biological systems are based on self-generated feedback and control and are not static, but evolving, adapting systems.* Biological systems work because of the self-regulating feedback and are, fundamentally, control systems. Feedback is the foundation of any biological system. Though current simulation techniques can handle feedback, the level of complexity and the pervasiveness of the feedback in biological systems is beyond what is commonly modeled in other more common model application areas. The modeling approach used in BioFusion, which generates a simulation over time, can handle such complexity.

4) *What is known about a particular biological process is often uncertain and contradictory.* The body of knowledge in a particular biological area will have gaps, as well as contradictions. Sources of information may only be tangentially related or may be unrepresentative studies that

provide insight without confirmation. Because of this, the research team must synthesize knowledge from a wide variety of sources to bring to bear on a modeling effort.

5) *The knowledge on which the model is built is constantly changing and evolving.* The biological sciences are constantly uncovering new information about disease processes. One of the goals of a BioFusion-based computational model is to synthesize that knowledge to express a comprehensive theory about the biological process under study. Once the model has been built representing the theory, the theory can be tested, refined, and validated. Because the model is modular and hierarchical, as new information emerges, it can be incorporated into the model.

As a result of these issues, not only is the actual modeling process difficult in a biological area, but the knowledge acquisition process is also highly complex. Thus, although lower level, highly specific areas can be handled using mathematical techniques, at the higher levels broader, less restrictive methods of representation are needed that allow for the complex feedback interactions and the large uncertainties in the knowledge. Modeling techniques that are forgiving of the lack and imprecision of knowledge are needed to effectively develop useful models in support of biological research. That is why we used a modeling technique developed specifically to model biological processes, namely BioFusion. This modeling approach was designed specifically to address the issues that make modeling biological systems complex, providing methods for knowledge acquisition, as well as flexibility in the implementation process to allow for experimentation and theory generation/exploration.

1.3 Overveiw of the BioFusion Modeling Technique

The BioFusion model development methodology was used to design, build, and validate a computer-based model of the laser effects on the RPEs in the eye. Though the model was developed such that it works under "non-stimulated" conditions as a baseline, it also includes the ability to provide certain exposures to the cellular environment, including laser exposure level, ambient temperature, oxygen availability, low-light exposure levels, and the current existing state of the cellular environment, including availability of various types of anti-oxidants. The PI for the work, Dr. Pamela Fink, developed the BioFusion methodology while at Interleukin Genetics, Inc. (formerly Medical Science Systems, Inc.). The technique received a patent on August 12, 1997 (patent number 5,657,255, "Hierarchical Biological Modeling System and Method"). Several papers have been published on this technique (Fink & Herren 1996, Fink & Herren 1997, and Herren et al. 1998) as well as on its use in a variety of applications (Fink et al. 2001, Fink & Morgan 2000).

The BioFusion model development methodology is a hierarchical, iterative approach to model development. It involves gathering together all of the knowledge that can be found on a particular targeted topic, organizing it into a coherent "theory" of the process in question, and implementing this "theory" into a computer-based model. The knowledge that is gathered can come from the literature, from experts, and from experiments that are run based on the need to acquire specific information in order to build the model. Thus, such models become a focal point for all that is known about an area, and an exploratory tool for gaining further understanding (Figure 1). BioFusion models can be used both to drive *in vitro* and *in vivo* experimentation as well as to serve as a repository for the results that are acquired from such experiments. In this way, the model can grow with the knowledge and continue to provide novel

insights and new ways of looking at the data, as well as help prioritize *in vitro* and *in vivo* studies.

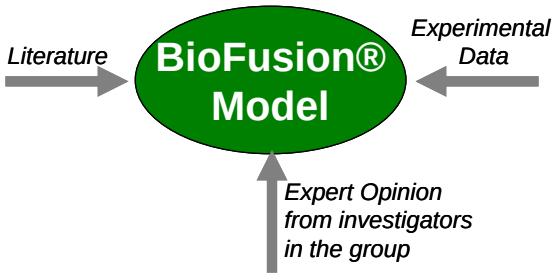

Figure 1 A BioFusion® model incorporates knowledge from literature, experimental data, and expert opinion from our investigators

The development of a BioFusion model involves a series of steps centered on the acquisition of the detailed knowledge required to build a model that exhibits the appropriate expected behaviors. The BioFusion model development process imposes a methodology on how to explore a given area targeted for modeling and how to analyze and synthesize what is found into a coherent picture, or theory, of the process being studied. A key attribute of a BioFusion model development effort is a series of meetings where the information that has been collected is sorted through and examined by a team of individuals involved with the development of the model. This team of individuals must include the experts in the field of application (such as the various aspects of cellular activity in the eye post exposure to a stimulus such as a laser), the individuals targeted as the end users of the model, and the computational modeling member(s) who will be implementing the computer model. These meetings are structured and managed by the Lead Computational Modeling team member who is expert in this knowledge acquisition process and who facilitates and directs the meetings to cover and analyze what is needed to build the model in the selected area in an orderly and rigorous manner.

Developing large, complex computer-based simulations, such as BioFusion models, is similar to developing large, complex, knowledge-based or intelligent systems. In both cases, very specific, detailed knowledge must be acquired and represented in a working software program, and the system behavior must be evaluated and accepted by an expert or set of experts. In both cases, the development of a working software system is more a problem of exploration than of implementation (Fink and Herren, 1997), because system requirements are not easily, clearly, or completely definable at the start. In such situations, specification and implementation are necessarily intertwined (Swartout and Balzer, 1982). The key methodology used in the development of knowledge-based or intelligent systems is the knowledge engineering process. The Principle Investigator on the project, Dr. Fink, has had extensive experience in the knowledge engineering process, and utilizes a software development methodology known as the Spiral Development Methodology (Figure 5), a common approach in software engineering of complex computer systems. The spiral software development process is highly iterative and capitalizes on the use of rapid prototyping to define the behavior of the final system. The width of the spiral at each point represents the size of the growing software system. Thus, the approach

advocates starting out small and adding capabilities incrementally as understanding of the problem increases.

Figure 2 demonstrates this interactive and iterative process. The general process consists of four major phases of development: acquisition, formalization, implementation, and verification/validation. As applied to simulation models, the development phases are knowledge acquisition, knowledge formalization, model implementation, and model behavior verification/validation. The iterative process used in BioFusion model development encompasses all four stages. At each point in a model development effort, the current focus defines what knowledge needs to be collected, formalized, implemented, and validated. The initial iteration generally involves only knowledge acquisition and formalization and produces a general description of the system and listing of the important components (e.g. the cells/molecules/behaviors involved in the biological process of interest). This information is documented in a set of knowledge diagrams. These knowledge diagrams illustrate graphically the relationships between important aspects of the biology being modeled. Depending on the targeted level of the model to be developed, the relationships may be between cells, between cells and various cytokines/biologically active molecules, or between various chemicals relevant to the key internal processes to the cell. In addition, all references used are documented in an electronic library.

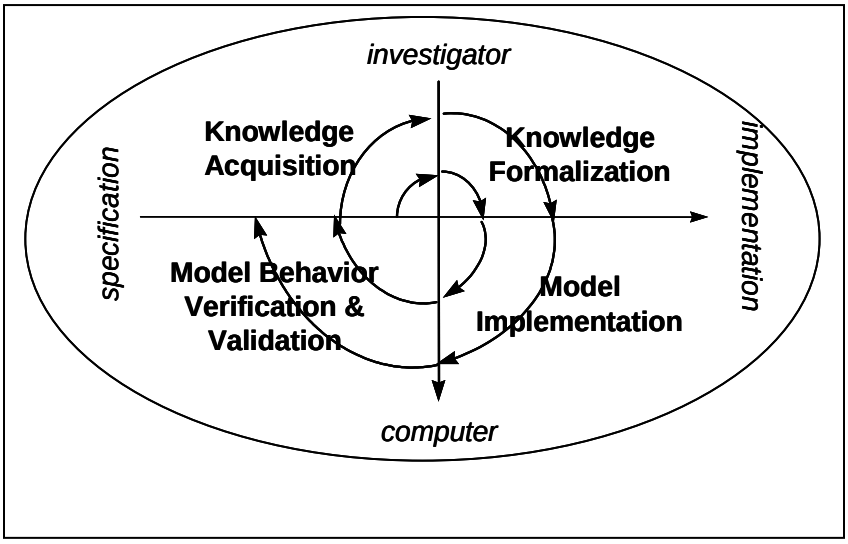

Figure 2 The spiral software development methodology used to develop BioFusion® models

Subsequent iterations in the process involve defining and building models of the components and then validating the behavior of each component in isolation. Later iterations involve defining and implementing interactions between components and validating the behavior of groups of components (e.g. how the cells interact based on physiological and anatomical characteristics, or how intra-cellular cascades interact or result in output from the cell). Final iterations involve global impacts on the system components and validating the correctness of the global changes across all components of the model. In each iteration, not only is the model expanded and/or refined, but the knowledge diagrams and the reference library are updated, as

well, to reflect the new knowledge/understanding. In this way a BioFusion model is fully documented at all levels.

The BioFusion approach to modeling biological systems can be implemented in a variety of ways. Usually, however, a standard commercial off-the-shelf (COTS) model development tool is used to generate the model. The COTS tool of choice has been a general modeling tool called Extend®, by Imagine That!, Inc. Supporting tools used for the knowledge diagrams and the reference library are Visio and EndNotes, respectively. These tools have worked very successfully on numerous other BioFusion modeling efforts and thus, this was the approach used in this project, as well.

2.0 SUMMARY OF WORK PERFORMED

A BioFusion model of the effects of lasers on the retina was developed for the Air Force Research Labs' Directed Energy Division. The work focused on the collection, analysis, organization, documentation, and implementation of information particularly on the retinal pigmented epithelial (RPE) cell, and its relationship to the photoreceptor cell.

Over the 24-month course of the project, the BioFusion modeling methodology was iterated through a number of times. Ultimately a model was developed that focuses primarily on the various oxidative and photochemical stress mechanisms that take place inside the RPE cell as a result of exposure to light. The model is primarily one of intracellular processes to the RPE cell, but with links to photoreceptor influences, as well as other environmental effects.

During the course of this project, a set of knowledge diagrams covering the processes involved with photochemical processes and oxidative stress in the RPE cells was developed, along with an electronic reference library. In total, 18 knowledge diagrams were developed and use for the model, along with two knowledge diagrams related to details on the visual cycle that were only used as a higher level source of knowledge for the model. Hundreds of research articles were collected in support of the knowledge acquisition process, but this number was narrowed down to approximately 250 that were actually used in the model development and recorded in the electronic reference library (using EndNotes). The rest of the papers collected and analyzed did not end up being in areas that became the focus for this research project, including RPE phagocytosis of photoreceptor outer segments, the interphotoreceptor matrix, and the visual cycle. A step between the reading of the reference papers and the development of the knowledge diagrams is the generation of relevant "factoids" about the various topics of interest. These are generated both as a direct result of reading the papers, as well as from meeting and discussing the information in the papers. These factoids are recorded in a set of Word documents that maintain the research "history" for the project. Approximately 2,500 such factoids were recorded in the process of developing the model for this project, covering topics ranging from photoinitiation and redox processes, to lipoprotein damage and oxygen control in the retina.

2.1 Overview of the Laser-Retina Model

Figure 3 provides a top-level knowledge diagram illustrating the areas covered in the the Laser-Retina model. This top level knowledge diagram shows the relationships between the major components of the model. Although the photoreceptor and visual cycle are illustrated in theis diagram, these areas were not implemented in the actual computer-based model in detail. Figure 4 is an example of a lower level knowledge diagram, one at the actual model implementation level. In particular, this knowledge diagram illustrates a portion of the reduction-oxidation processes that are significant to the RPE cell's response to laser exposure. The model contains a total of 18 diagrams, including ones for photoinitiation, the melanin effect, antioxidant enzymes, the reduction chain, metabolism, lipid peroxidation, DNA damage, and the visual cycle, among others. The emphasis of the knowledge content is on oxidative processes because they are instrumental to the photoreceptor and RPE cells' response mechanisms to light exposure.

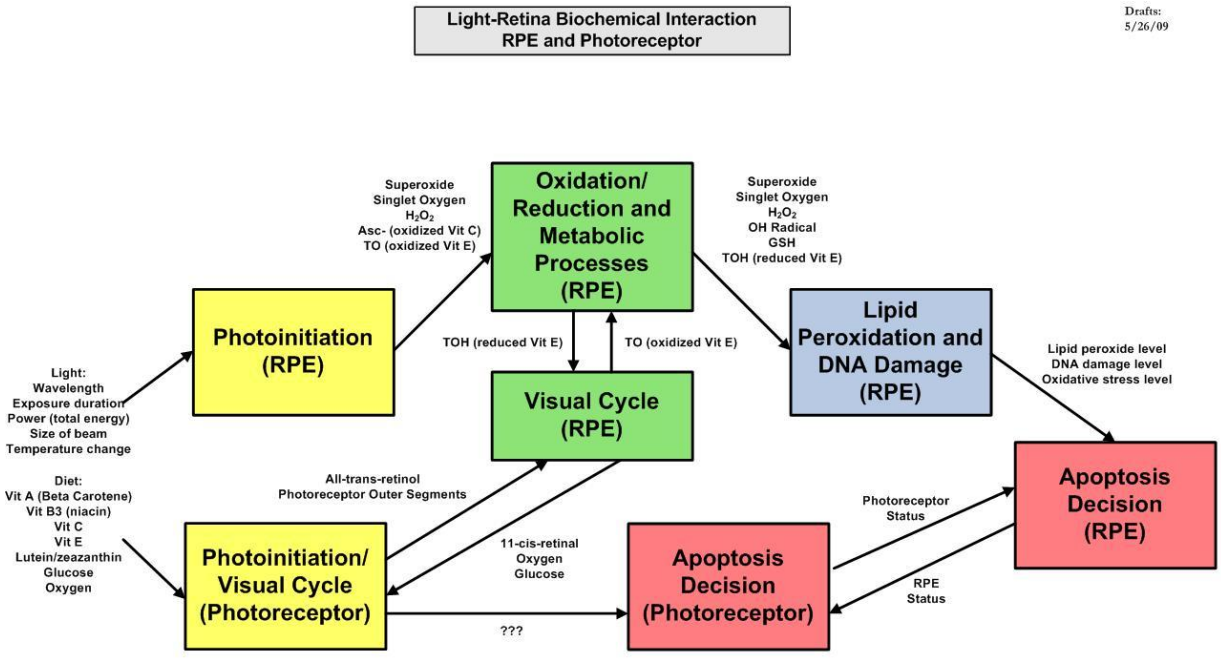

Figure 3 The top level knowledge diagram for Laser-Retina Model

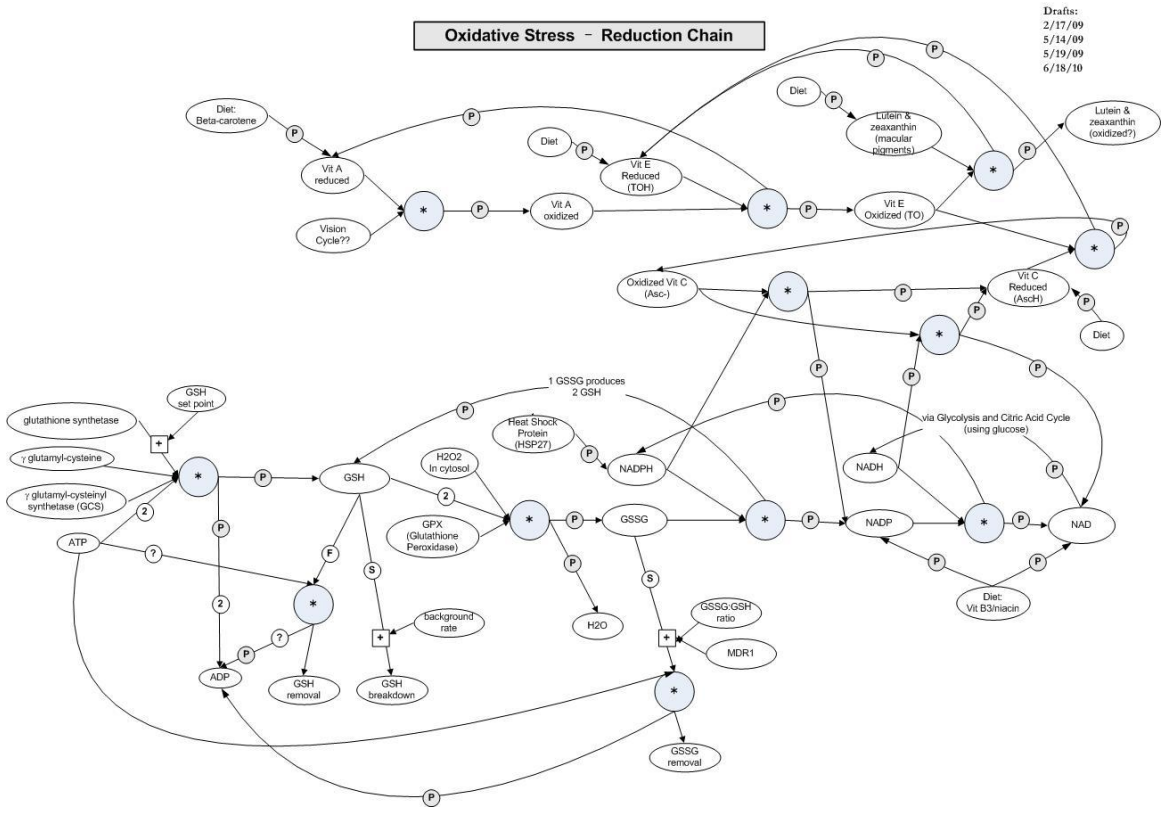

Figure 4 An example lower level knowledge diagram for the Laser-Retina Model

Figure 5 provides an example of what the Laser-Retina Model looks like. As mentioned previously, the model is implemented in a software development tool called ExtendSim, by Imagine That!, Inc. This tool is a visual model development tool where the modeling blocks can be customized to meet specific modeling needs. Figure 5 illustrates the hierarchical nature of the Laser-Retina model, which is supported by the ExtendSim model development tool. The figure shows the top level of the model, along with the next lower level of the model for the Redox Chain, and then further details for the GSH/GSSG portion of the redox chain.

To run a simulation, attributes of a light exposure, including power, spot size, wavelength, and duration, is entered. The model is designed initially to run in a balanced state with nominal visible light exposure. Figures 6 and 7 show, respectively, a portion of the input screen and a few resulting charts from a simulation run where not only ordinary ambient, visible

light exposure is input, but also a laser pulse of 10 W, with laser diameter (spot size) of 1 cm, and exposure duration of 10 seconds is introduced at timestep 500. A timestep within the model is set to be equivalent to one second real clock time. The model is designed to run for the equivalent of 48-72 hours (2880 - 4310 minutes, or 172,800-258,600 seconds), but it can be set to run for any duration. Actual model run time for a forty-eight hour simulation takes about 6-7 seconds on a standard Windows PC.

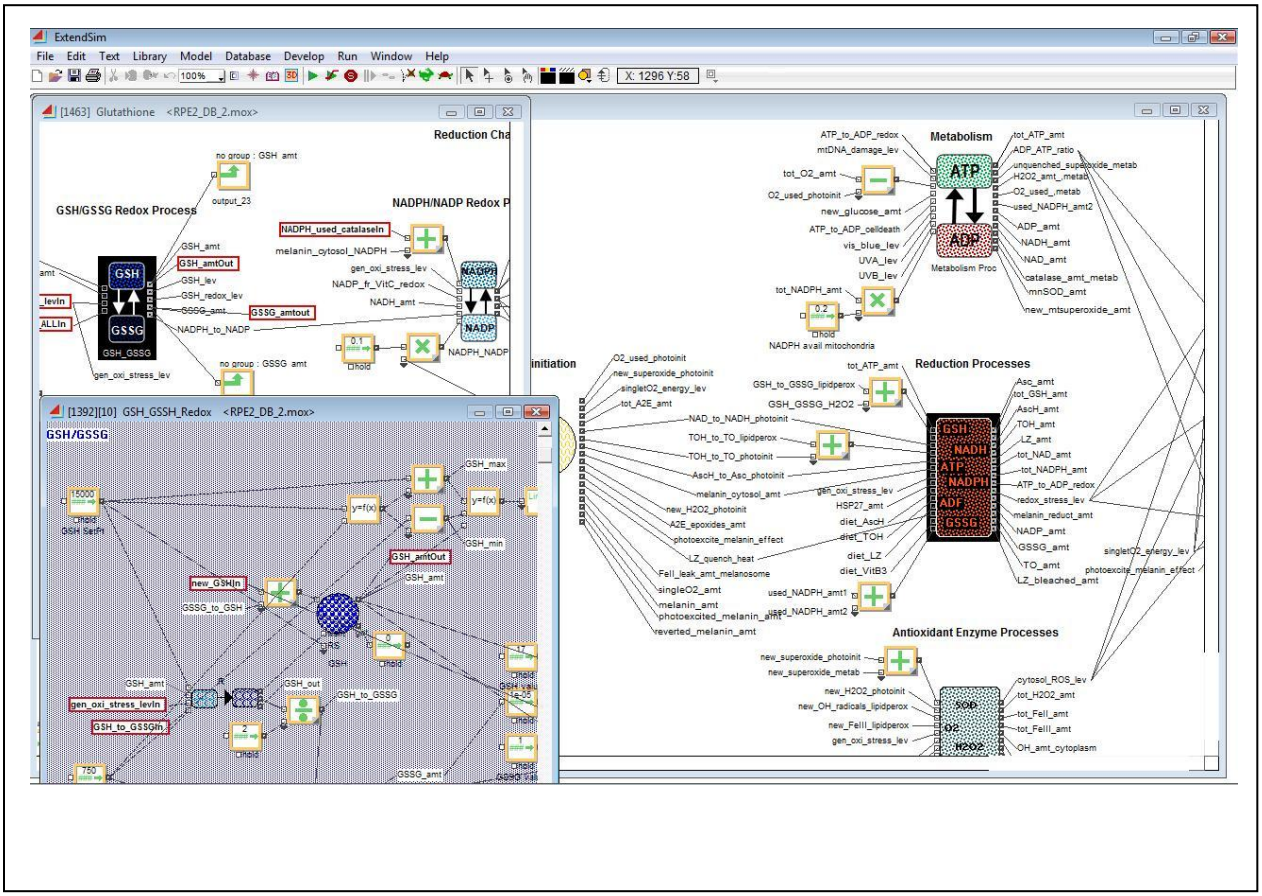

Figure 5 An example portion of the Laser-Retina Model showing the hierarchical nature of the model

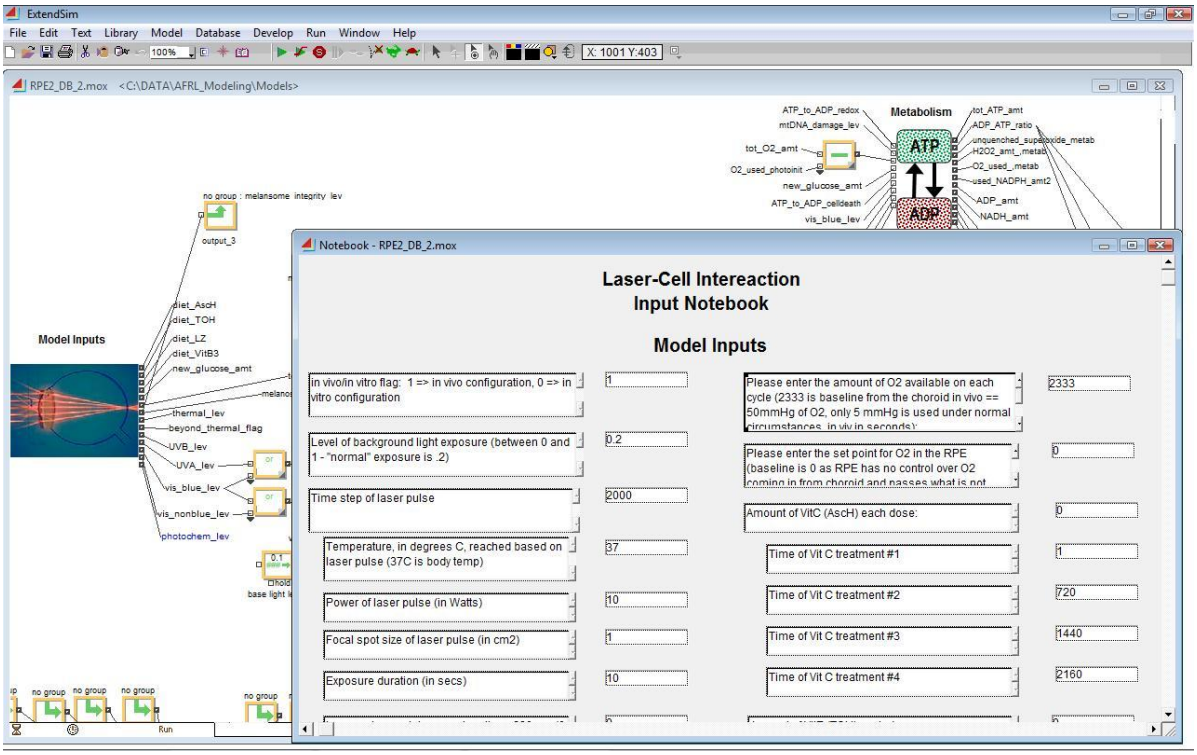

Figure 6 A portion of the input screen for the Laser-Retina Model

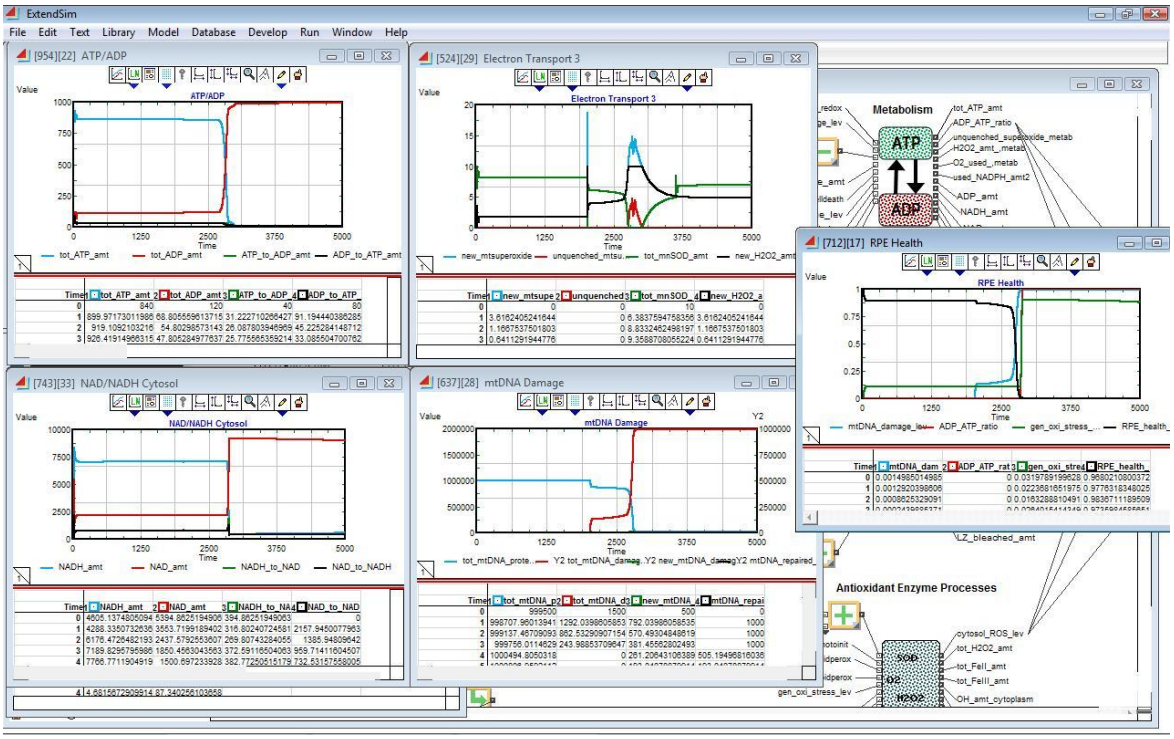

Figure 7 Some example output charts of the results of running the Laser-Retina Model

The effect of the laser pulse input can be seen in the five charts shown in Figure 7. The charts (from upper left, and across to lower right) present the modeled response in the RPE cell of Vitamin C (asc/ascH), some reactive oxygen species (including superoxide, hydrogen peroxide, and the hydroxyl radical), NAD/NADH, and glutathione (GSH/GSSG) to the laser exposure. A portion of the RPE Cell Health chart, which provides a more global assessment of the RPE cell's stress level, is also visible on the right. As can be seen in these charts, the model is stable prior to the treatment, has a response for approximately two to two and half hours post-treatment, and then re-stabilizes. Thus, this level of treatment produces some damage to the RPE cell, but not enough to actually kill it.

The goal of the Laser-Retina model was to develop a tool that could be used to help explore the mechanisms by which an RPE cell becomes damaged and/or dies due to light exposure. There are some data in the literature regarding laser-tissue interactions *in vitro* and even though there appears to be some correlation with animal studies (Denton et al., 2008, Schulmeister et al., 2008), direct extrapolation of the *in vitro* data would be problematic at present. We have designed the model to allow for bifurcated configurations for *in vitro* and *in vivo* environments. In the retina, RPE cells have a cuboidal shape with the melanosome particles layered just beneath the microvilli at the apical surface. The *in vitro* configuration excludes the ocular layers anterior to the RPE layer that are considered as optical filters in the *in vivo* configuration, and it accounts for the flattened, fried egg-like shape of RPE cells grown on plastic as monolayers. This flattened shape exposes cellular organelles such as the nucleus and the mitochondria to greater doses of incident light as compared to the RPE cell layer *in vivo* for a

given laser power density. Additionally, the *in vitro* scenario does not account for the complex relationships between retinal RPE cells and the photoreceptor cells that they service, such as providing needed nutrients, removing waste products, recycling retinal via the visual cycle, and phagocytosing shed outer segments. It is possible that the mechanisms that damage and/or kill an RPE cell in one environment are quite different from those that damage and/or kill it in the other. The model should help to delineate these issues.

2.2 Overview of the Model Database and Interface

The Laser Retina Model contains approximately 40 inputs and 50 outputs, representing the various molecules or ratios that are tracked by time step throughout the course of a model run. As a result, thousands of data values are produced and recorded during each simulation run. These data are initially only available within the ExtendSim modeling and simulation environment, which only stores the results from a total of four simulation runs at a time in chart form and only the most recent run on table form with actual values. Older results are dumped in chronological order as new simulation runs are made. In order to make data available for analysis to scientists performing research beyond these few simulation runs, the data must be exported from the Laser-Retina Model and stored into a database for later access. Thus, a database and user interface was developed to support this need. This "reporting" system offers a customizable interface to the user so that the user can easily select the data they need to create a report in the desired report format. This customizable report format allows for the selection of graphs/charts, if wanted, or just numerical tables. The structure of the report system includes three main parts, 1) the data source and import facility, 2) a customization interface, and 3) the reports.

The data source is the Laser-Retina Model itself. Each simulation run produces a pipe-delimited (e.g. "|") text file containing the value of all inputs, and the values of all molecules available in the model on each time step as output. Each file generated is named based on a root name and a sequence number so that the results of each simulation run is stored uniquely and not written over. Each of these files can then be imported into the model reporting tool that provides a database for storage and an interface for selecting and viewing the model run data. The model database and reporting tool is implemented in Microsoft Access. Figure 8 provides an example of the contents of the exported model data file; Figure 9 shows the first screen of the model database, where the file can be imported into the database, and the resulting imported data; and Figure 10 shows the user can select the particular simulation run values to view. Figure 11 shows a resulting graph that plots the data across time from the simulation run. This chart is similar to the ones produced within the ExtendSim model, but are based on a sampling of the data every minute instead of every second to save storage space.

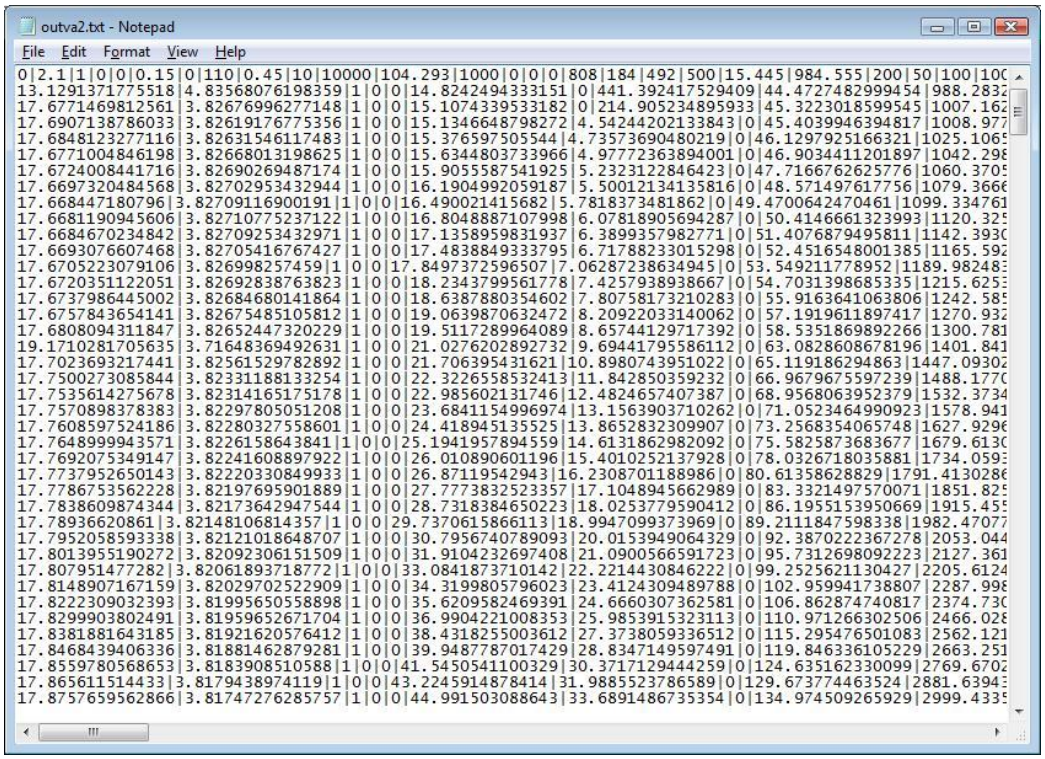

Figure 8 Example contents of the text file output from the Laser-Retina Model

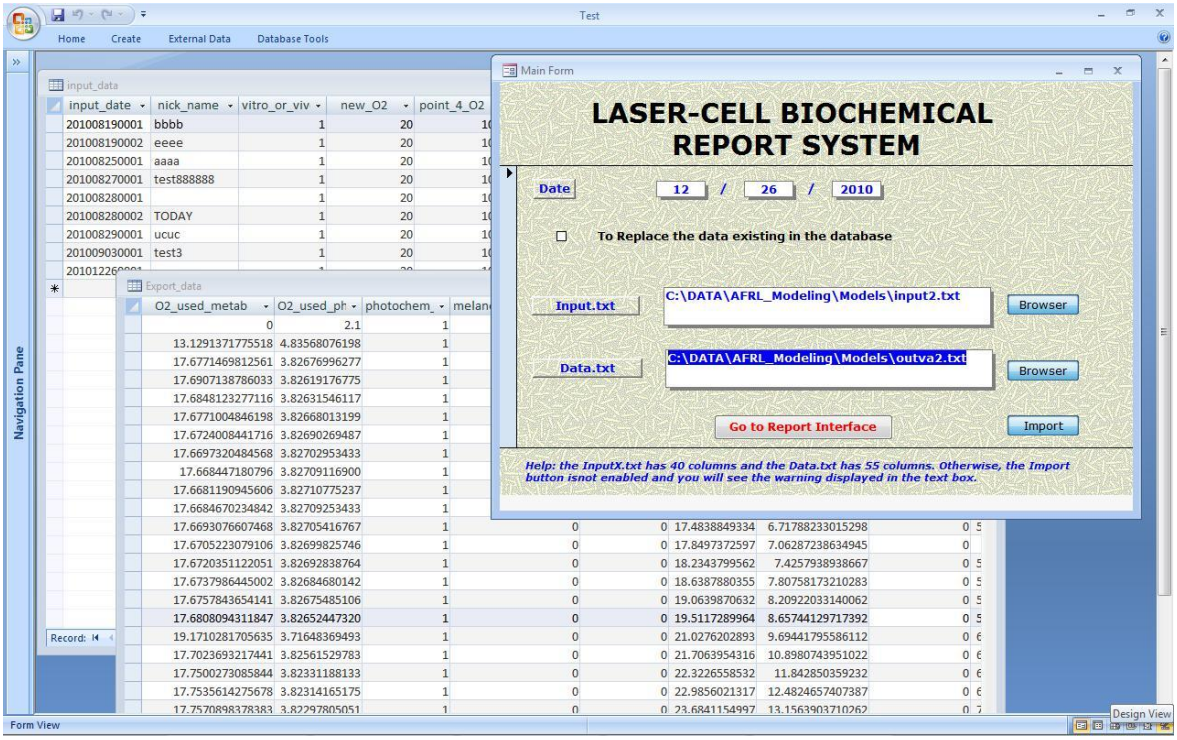

Figure 9 Interface for importing simulation data into the model database and resulting data table

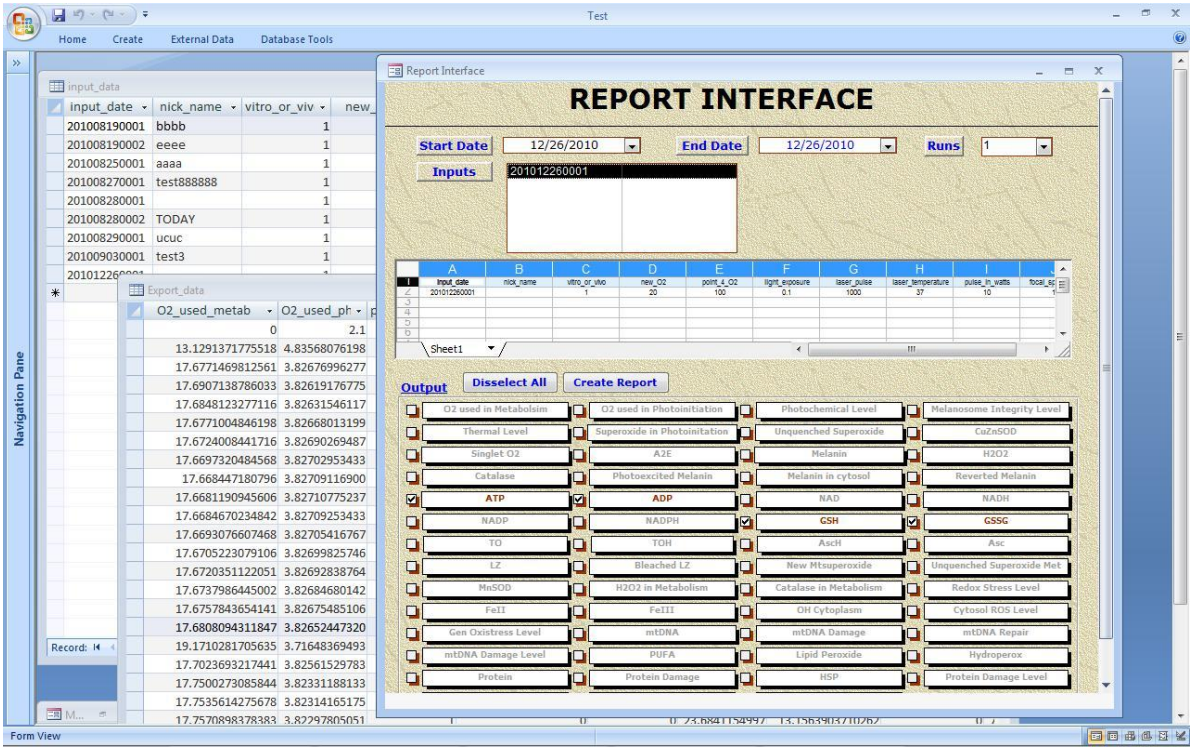

Figure 10 Interface for selecting data from the model database to present in a chart or charts

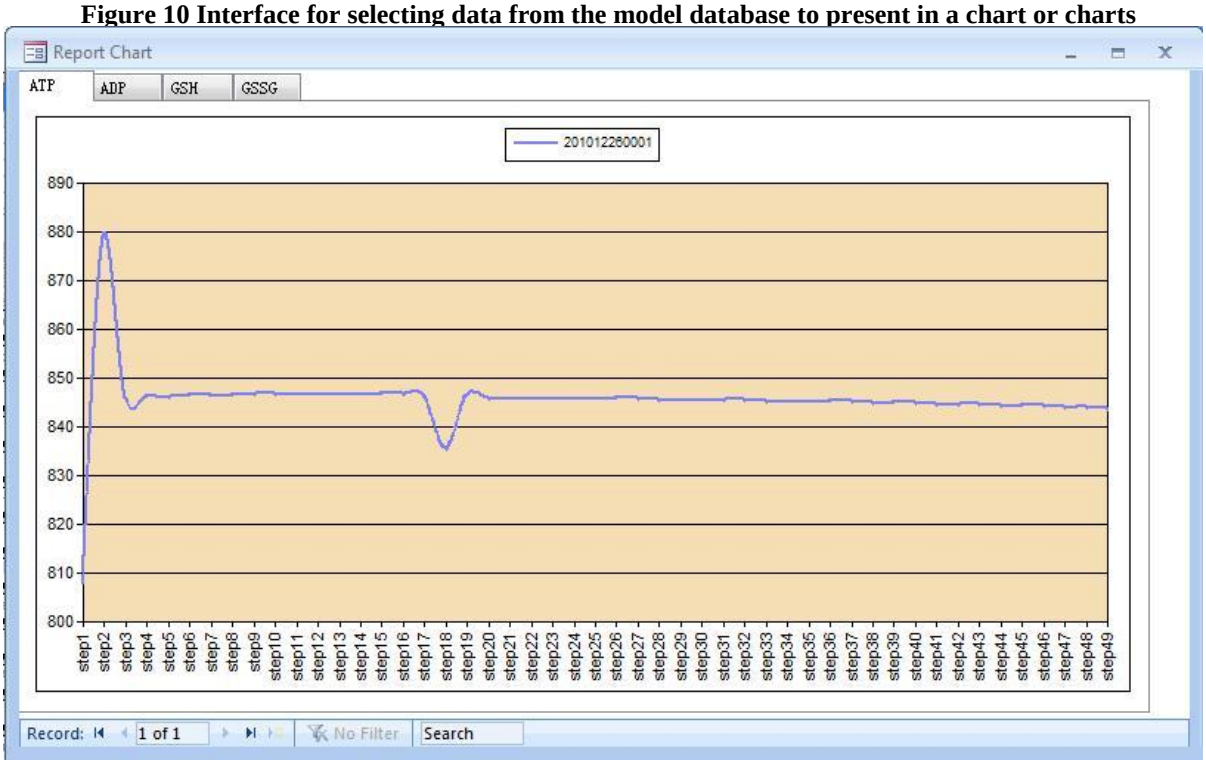

Figure 11 An example chart of selected data from a Laser-Retina Model simulation run

3.0 CONCLUSIONS

The BioFusion modeling approach: 1) is hierarchical, 2) is modular, 3) can model sufficient depth, 4) can model sufficient breadth, and 5) is synergistic. This technique allows for sufficient depth at the cellular and sub-cellular levels to model potential interventions at their source, while at the same time covering sufficient breadth of the relevant biology to model events at the organ, system, and organism levels. Because of the hierarchical and modular nature of the resulting models, the simulations are sufficiently complex to allow for many new, unique, and revolutionary insights into the biology being explored. BioFusion models can be used both to drive *in vitro* and *in vivo* experimentation as well as to serve as a repository of results found in the literature. In this way, the model can grow with the knowledge base and continue to provide novel insights into mechanisms and interactions among cells and systems.

The project reported on here has addressed a key component of the retina, namely the photoinitiation and oxidative stress response of the RPE cell to laser exposure, as the starting point for further understanding of how light affects the eye. Use of the modular, hierarchical approach to developing the model will allow work to focus on the exploration of the effects on the RPE cell's viability initially, and later to expand to include details of other cell types and processes found in the eye, in response to light exposure.

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APPENDIX A – EndNotes Reference Library

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APPENDIX B – Meeting Notes and Factoids

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APPENDIX C – Knowledge Diagrams

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APPENDIX D – BioFusion Model

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APPENDIX E – BioFusion Database

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APPENDIX F – Publications and Presentations

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